SOIL CHEMISTRY

Changes in the Carbon and Nitrogen Isotopic Composition of Organic Matter in Soils of Different Thermal Stability after Free-Air CO_2 Enrichment for Three Years

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Received February 13, 2007; in final form, September 20, 2007

Abstract—The hypothesis that the biological availability of soil organic matter (SOM) pools is inversely proportional to their thermal stability was tested using the isotopic difference between the atmospheric CO_2 ($\delta^{13}C = -8.0‰$) and ^{13}C-enriched CO_2 ($\delta^{13}C = -47‰$) fertilizers, as well as ^{15}N-labeled fertilizers. The soil samples from spring wheat plots subjected to treatment with ambient (370 ppm) and elevated (540 ppm) CO_2 concentrations for three years were analyzed by the thermogravimetric method. Based on the weight loss, five SOM pools were distinguished where the total C and N contents and isotopic compositions ($\delta^{13}C$) and ($\delta^{15}N$) were determined. The contents of new C and N and their mean residence times in pools were calculated. The incorporation of ^{13}C and ^{15}N and their turnover rates did not depend on the thermal stability of the SOM pools, which disproved the hypothesis being tested.

INTRODUCTION

The artificial enrichment of the open atmosphere with carbon dioxide (free-air CO_2 enrichment, FACE) is widely used for studying the transformation of C and N in soil organic matter (SOM) [22, 26, 32]. In distinction from the closed chamber method, the FACE method neutralizes the formation of a microenvironment and allows studying the natural processes occurring under field conditions [43]. The enrichment of the atmosphere with CO_2, whose isotopic composition can vary from –35 to –50‰, and the use of ^{15}N-labeled fertilizers ($\delta^{15}N \approx 350‰$) allow the transformation of C and N in the soil to be traced from the changes in $\delta^{13}C$ and $\delta^{15}N$ in the SOM [17, 33, 43, 44]. The differences in the isotopic composition of the SOM between the control plots and the plots with elevated CO_2 concentrations and/or applied fertilizers, as well as the duration of the experiment, are the key parameters for calculating the contents of substituted C and N and their turnover rates in the soil [2].

Different fractionation methods are widely used for the more thorough study of C and N transformation in the SOM. Most of these methods, e.g., SOM fractionation by density [18, 31], particle size [21, 30], or extractability [8], can be combined with isotope analysis methods.

A method of SOM fractionation is based on the thermal decomposition stability of the SOM [24, 27, 29, 35, 39, 40]. A gradual increase in the temperature entails the decomposition (mainly oxidation) of organic and inorganic soil components in accordance with their thermal stability. The gradual heating of samples with the simultaneous measurement of the weight loss is called thermogravimetry (TG) [1, 18].

TG finds wide use in many fields of soil science. Schulten and Leinweber [37] studied the properties of organic–mineral complexes; Provenzano and Senesi [36] studied humic substances; Lopez Capel et al. [29], Francioso et al. [11], and Plante et al. [35] used the TG method to study the humification of SOM. Siewert [39] studied the thermal stability of soils in different temperature ranges and the emission of CO_2 from soil under incubation; he advanced the hypothesis that a correlation exists between the thermal decomposition stability of the SOM pools and their availability for microbial degradation.

Although TG is a rapid method of determining changes in the SOM quality, it was not used to study the changes in organic matter of soils subjected to an elevated concentration of CO_2 in the atmosphere.

The aim of this work was to study the thermal properties of the organic matter in a soil subjected to ambient and elevated CO_2 concentrations for three years and to test the hypothesis of the relationship between the thermal stability and biological availability of SOM by comparing the $\delta^{13}C$ and $\delta^{15}N$ in SOM pools decom-

posed at different temperatures. If the hypothesis is true, the SOM pools decomposed at lower temperatures will have $\delta^{13}C$ values close to the $\delta^{13}C$ of plants growing at elevated CO_2 concentrations and the $\delta^{13}C$ of the SOM mineralized at high temperatures will be closer to the $\delta^{13}C$ of pools in the soil occurring at the ambient CO_2 concentrations. The $\delta^{15}N$ values of SOM fractions from the soil with a low thermal stability will be close to the $\delta^{15}N$ of the fertilizers applied, and the mean residence time (MRT) of C and N in the SOM pools with a low thermal stability will be lower than that in the pools resistant to thermal decomposition.

EXPERIMENTAL

Objects and Methods

The FACE experimental plot is located in Hohenheim, Baden-Württemberg, Germany (48°43′ N, 9°13′ E). The climate of the region is temperate with a mean average annual temperature of 8.7°C and precipitation of 680 mm/year (from the data of the Stuttgart–Hohenheim meteorological station for 1961–1990). The FACE experiment was started in 2002 on 15 plots: 5 plots with the elevated CO_2 concentration in the atmosphere (540 µmol/mol, $\delta^{13}C = -47‰$), 5 plots with the ambient CO_2 concentration (370 µmol/mol, $\delta^{13}C = -8‰$), and 5 control plots with the ambient CO_2 concentration but without protection maintaining the specified CO_2 concentration. On all the plots, spring wheat was planted annually in combination with some weeds typical of the agricultural lands in Germany. Since 2003, inorganic NPK fertilizers have been applied annually at 140 kg N, 30 kg P, and 60 kg K per hectare to all the plots with the ambient and elevated CO_2 concentrations. For the KNO_3 applied, $\delta^{15}N = 333.75‰$ [9].

The soil is a carbonate-free Gleyic Cambisol [45] containing 9, 22, and 69% sand, clay-silt, and clay fractions, respectively. The bulk density of the 0- to 10-cm layer is 1.4 g/cm^3, the pH is 6.8, the C_{org} is 1.45%, the N_{tot} is 0.16%, and C/N = 9.1.

Soil samples for the thermogravimetric analysis of the $\delta^{13}C$ and $\delta^{15}N$ were taken from the depth of 0–10 cm with a soil sampler (inner diameter 5 cm) in October 2005. The samples were dried at room temperature and sieved through a sieve with a mesh diameter of 2 mm. All the visible roots and plant residues were thoroughly removed with tweezers. A soil sample (10 g) was ground to powder in an MM2 ball mill (Fa Retsch) for 15 s.

The thermogravimetric analysis was performed using a Netzsch STA 409EP instrument with a TASC 414/3 module (Fa Netzsch). A soil sample of 0.1 g was placed in an aluminum crucible and heated from 20 to 1000°C at a rate of 2°C/min under an airflow of 1 ml/min. The weight of the soil sample was measured every 10 s. Calcined kaolinite preliminar-

ily ignited at 1250°C was used as the control material [1].

Based on the weight loss curves obtained from the thermogravimetric analysis, the threshold temperatures (200, 310, 400, and 480°C) were determined. Then, all the soil samples were divided into four groups, and each of them was ignited at these threshold temperatures in a Heraeus MR-260 muffle oven. The ignition conditions (the heating rate, the initial sample weight, and the blowing rate) were similar to those of TG. After ignition, the soil samples were cooled to room temperature, thoroughly mixed, and used for the mass-spectrometric analysis.

For the $\delta^{13}C$ and $\delta^{15}N$ analysis, 10 to 40 mg of soil ignited in the muffle to a threshold temperature was placed in a tin foil capsule. The contents of C and N and their isotopic compositions were determined using a Delta Plus XP mass spectrometer (Thermo Finnigan, Bremen) connected to a Euro EA C/N analyzer (Eurovector Instruments and Software, Hekatech GmbH, Wegberg). Acetanilide was used as an internal standard for the $\delta^{13}C$ and $\delta^{15}N$ analysis. The final isotopic composition was expressed as $\delta^{13}C$ versus the Pee Dee Belemnite (PDB) international standard ($^{13}C/^{12}C = 0.0112372$) for C and as $\delta^{15}N$ related to the atmospheric N_2 ($^{15}N/^{14}N = 0.0036765$) for N.

The $\delta^{13}C$ and $\delta^{15}N$ values for each SOM pool were calculated from the isotope mass balance equation [3]:

$$M_{tut}\delta_{tut} = M_{fr1}\delta_{fr1} + M_{fr2}\delta_{fr2} + \ldots + M_{fr5}\delta_{fr5}, \quad (1)$$

where M_{tot} is the total content of C or N in the sample; δ_{tut} denotes the $\delta^{13}C$ or $\delta^{15}N$ values of the SOM before heating; and M_{fr1}, M_{fr2}, … M_{fr5} denote the contents of C or N in the SOM pools after ignition at the threshold temperatures.

The portion of C substituted in the SOM during the FACE experiment (C_{FACE}) was calculated according to Balesdent and Mariotti [3]:

$$C_{FACE} = (\delta^{13}C_{high} - \delta^{13}C_{atm})/(\delta^{13}C_{theor.high} - \delta^{13}C_{atm})100, \quad (2)$$

where $\delta^{13}C_{high}$ is the value of the SOM $\delta^{13}C$ at the elevated CO_2 concentration, $\delta^{13}C_{atm}$ is the value of the SOM $\delta^{13}C$ at the ambient CO_2 concentration, and $\delta^{13}C_{theor.high}$ is the theoretical $\delta^{13}C$ value of the SOM formed under a permanently high CO_2 concentration. This approach to the FACE method uses the theoretical isotopic composition of the SOM formed with CO_2 whose isotopic composition differs from that in the free air. The values of the theoretical isotopic composition of the SOM were derived from the $\delta^{13}C$ of plants growing at elevated CO_2 concentrations by subtracting the difference between the $\delta^{13}C$ of plants growing at the ambient CO_2 concentration and the $\delta^{13}C$ of the corresponding SOM pool occurring at the ambient CO_2 concentration.

The content of new N incorporated into the SOM during two years of fertilization was calculated analogously:
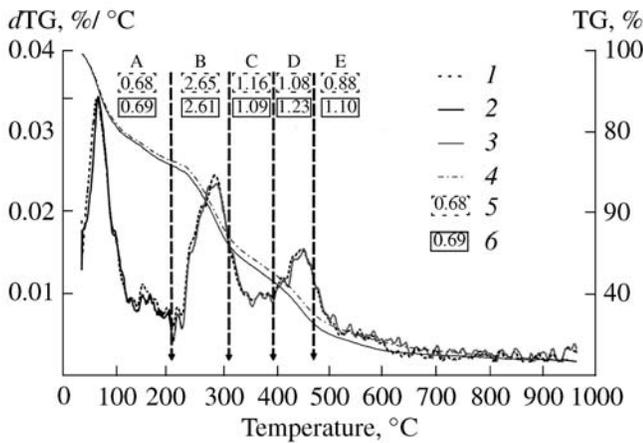
Fig. 1. Thermogravimetry and differential thermogravimetry of soil samples in the treatments with the ambient and elevated CO_2 concentrations. The total weight losses in the TG analysis equal to 6.44 and 6.71% for the treatments with the ambient and elevated CO_2, respectively, were taken as 100%. (*1*) dTG of the ambient CO_2; (*2*) *d*TG of the elevated CO_2; (*3*) TG of the ambient CO_2; (*4*) TG of the elevated CO_2, % of the initial value; (*5*) weight losses upon the ambient CO_2, % of the initial value; (*6*) weight losses upon the elevated CO_2 concentration, % of the initial value; the arrows denote the threshold temperatures used for the SOM fractionation.

$$N_{fert} = (\delta^{15}N_{soil,act} - \delta^{15}N_{soil,ini})/(\delta^{15}N_{fert} - \delta^{15}N_{soil,in})100, \quad (3)$$

where $\delta^{15}N_{soil,act}$ is the SOM $\delta^{15}N$ after fertilization for two years, $\delta^{15}N_{soil,ini})$ is the SOM $\delta^{15}N$ before fertilization; and $\delta^{15}N_{fert}$ is the fertilizer $\delta^{15}N$.

The values of C_{FACE} and N_{fert} were calculated for the whole soil and each SOM pool.

The mineralization rate (MR) for the C_{FACE} and N_{fert} in the SOM was calculated using a first-order equation [3]:

$$CM = -\ln(1 - M/100)/t, \quad (4)$$

where M is the content of C_{FACE} or N_{fert} (Eqs. (2) and (3), respectively) in the SOM, and *t* is the time of impact of the elevated CO_2 concentration on the plants and soil and the time of fertilization in the experiment.

The mean residence times (MRT) of C_{FACE} and N_{fert}, which are indicative of the C and N availability for plants, were calculated as the inverses of the decomposition rates [14].

The experiments were carried out in five replicates for the treatment with the elevated CO_2 concentration and in four replicates for the treatment with the ambient CO_2 concentration. The significance of the differences between the $\delta^{13}C$ and $\delta^{15}C$ and between the C and N contents in the different SOM pools was tested by the two-way analysis of the variance (ANOVA) using Statistica 5.0 software (StatSoft). The primary statistical processing of the data was performed using Microsoft Excel 2003 (Microsoft Corp.).

Experimental Procedure

Thermogravimetric analysis of the soil. The thermogravimetric data are presented in two ways: (1) cumulative weight loss curves for the soil samples (TG, %) and (2) differential weight loss curves of the soil samples per one degree of temperature rise (*d*TG, %/°C) (Fig. 1). No reliable differences in the soil weight loss were observed between the treatments with the ambient and elevated CO_2 concentrations; therefore, identical threshold temperatures of the thermal fractionation were selected for both treatments.

Based on the *d*TG curves, five temperature ranges were defined: three of them included the maximum weight losses of the soil samples during ignition (A, 20–200; B, 200–310; and D, 400–480°C); the E range (480–1000°C) mainly included the mineral residue; and the C range (310–400°C) was defined to separate the B and D ranges, which included different organic compounds. The boundaries of the ranges (the dashed arrows in Fig. 1) denoted the threshold temperatures to which the soil samples were heated to analyze them for the total C and N and their isotopic compositions. The heating of the samples to 200°C removed the water and the organic substances decomposed at this temperature, and the soil residue with the more thermally stable substances was further analyzed. The other SOM pools were separated in a similar way. Then, taking into consideration the weight losses and the measured values of the C and N contents and $\delta^{13}C$ and $\delta^{15}N$ in the residue, the corresponding values were calculated for the intermediate SOM pools decomposed in the ranges of 200–310, 310–400, and 400–480°C.

The mean values of the weight loss for the five SOM pools in the treatments with the ambient and elevated CO_2 concentrations did not differ reliably: 6.44 ± 0.18 and $6.71 \pm 0.23\%$, respectively. The largest weight losses were observed in the B range: the weight of the soil samples with the ambient CO_2 concentration decreased by $2.65 \pm 0.02\%$, and the weight of the soil samples with the elevated CO_2 concentration in the atmosphere decreased by $261 \pm 0.03\%$. The smallest weight losses of the SOM were observed in the A range (up to 200°C): 0.68 ± 0.04 and $0.69 \pm 0.02\%$ for the treatments with the ambient and elevated CO_2 concentrations, respectively.

The highest contents of C and N were found in the SOM pool decomposed at 200–310°C, which corresponded to the maximum weight losses. The lowest contents of C and N were in the pool mineralized in the last (E) temperature range (Fig. 2). The analysis of the variance showed that the increase in the CO_2 concentration had no effect on the ignition weight loss and the carbon content in the SOM pool, except for the pool mineralized in the C range. A reliable difference in the N content was found between the SOM pools decomposed at 310–400 and 400–480°C. In distinction from the CO_2, the effect of temperature on the content of C and N was reliable for all the SOM pools.
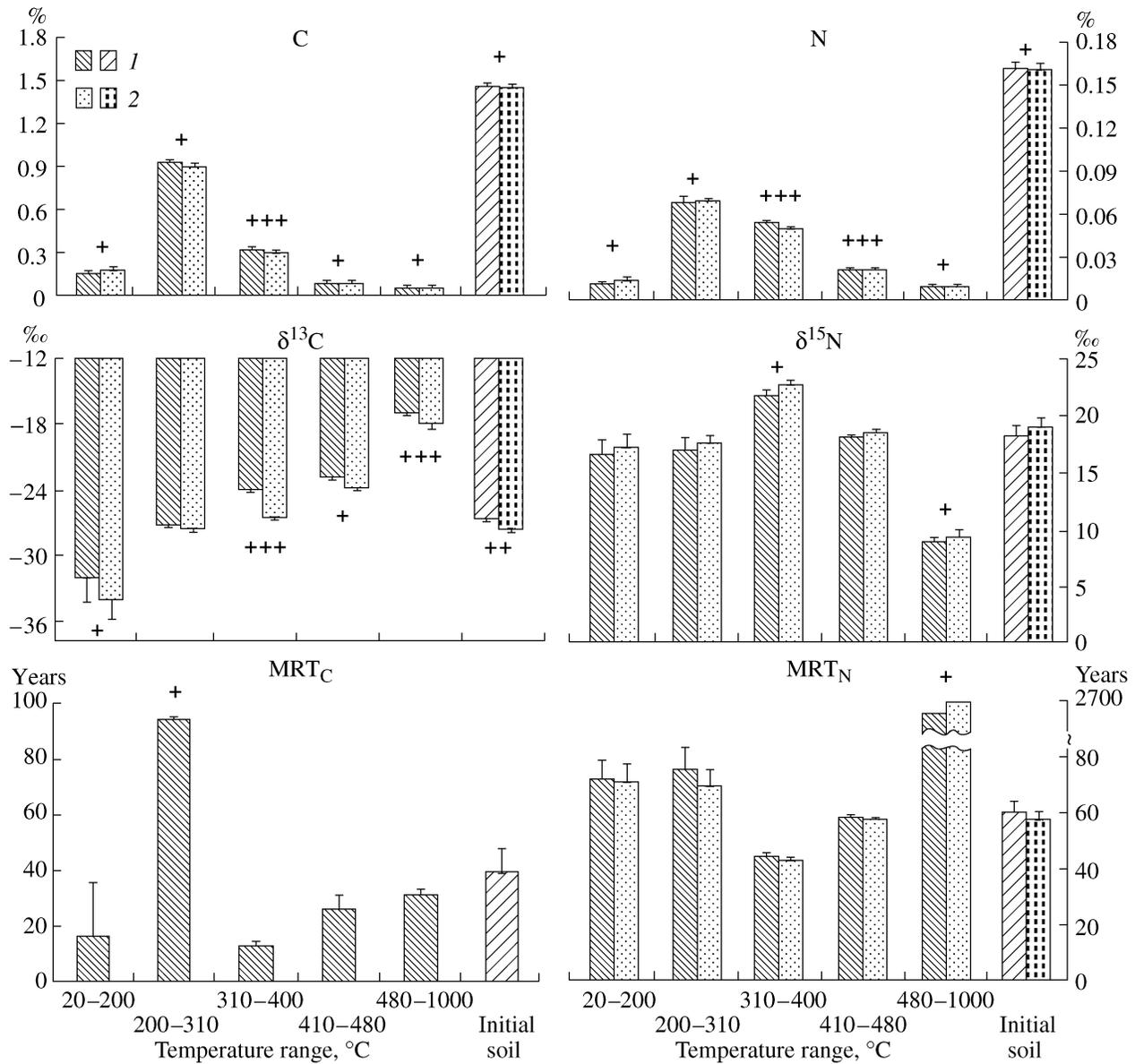
Fig. 2. Carbon and nitrogen contents, $\delta^{13}C$ and $\delta^{15}N$ values, and the mean residence times of C (MRT_C) and N (MRT_N) in the initial soil and SOM pools with different thermal decomposition stabilities under (*1*) the ambient and (*2*) elevated CO_2 concentration in the atmosphere. The reliability of the difference between the values ($P = 0.05$) is indicated by the signs "+," "++," and "+++" for the temperature, CO_2, and both parameters, respectively.

C and N isotopic compositions in SOM pools. The impact of the elevated CO_2 concentration in the atmosphere for three years decreased the SOM $\delta^{13}C$ value. Reliable differences in the isotopic composition of the C were observed between the treatments in the SOM pools mineralized in the C and E ranges; the differences between the corresponding SOM pools in the A, B, and D ranges were unreliable ($P > 0.05$). The highest depletion of ^{13}C was found in the pool decomposed at 20–200°C: the soil had $\delta^{13}C = -32.32 \pm 2.15‰$ in the treatment with the ambient CO_2 concentration and $\delta^{13}C = -34.32 \pm 1.85‰$ in the treatment with the elevated CO_2 concentration. The effect of the temperature

increased the $\delta^{13}C$ values in the SOMs of both treatments. This was due to the ^{13}C isotope fractionation during the slow decomposition, when the compounds with the light ^{12}C isotope were decomposed to CO_2 more rapidly than the compounds with the heavy ^{13}C isotope. As a result, the thermally stable SOM pools were enriched with ^{13}C [29].

The $\delta^{15}N$ values of the organic matter in the initial soil, which was not subjected to the thermal treatment, and those of the SOM pools mineralized in the A, B, and D ranges were similar and equal to 18.00‰. Reliably higher $\delta^{15}N$ values were obtained for the SOM pool decomposed at 310–400°C: 21.69 ± 0.43 and
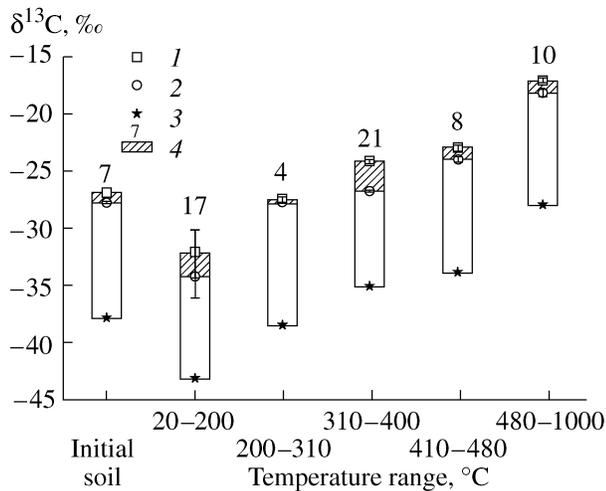
Fig. 3. The values of $\delta^{13}C$ ($\pm$ standard error) and the C_{FACE} content (%) in the initial soil and SOM pools with different thermal decomposition stabilities. The theoretical $\delta^{13}C$ values of the soil under a permanent elevated CO_2 concentration were used to calculate the C_{FACE} portion in the SOM. (*1*) Soil under the ambient CO_2 concentration; (*2*) soil under the elevated CO_2 concentration; (*3*) soil under the theoretical CO_2 concentration; (*4*) C_{FACE} portion in the SOM, %.
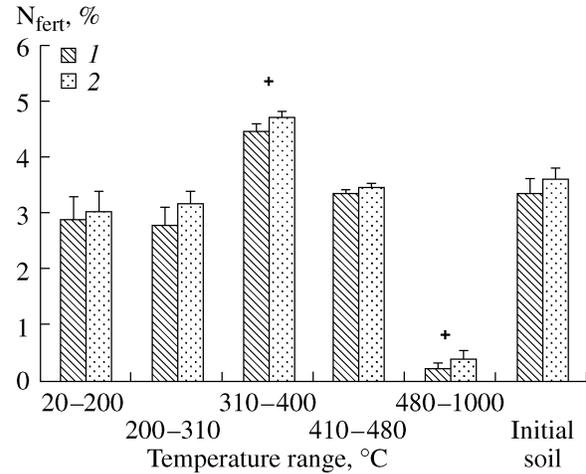

Fig. 4. Contents of N_{fert} ($\pm$ standard error) in the SOM of the initial soil and pools with different thermal decomposition stabilities after fertilization at 140 kg/ha annually for two years. The reliability of the difference between the values ($P = 0.05$) is indicated for the temperature by the sign "+." The CO_2 had no statistically significant effect. (*1*) Ambient CO_2 concentration; (*2*) elevated CO_2 concentration.

22.68 $\pm$ 0.30‰ for the treatments with the ambient and elevated CO_2 concentrations, respectively. The lowest $\delta^{15}N$ values (8.91 $\pm$ 0.37 and 9.37 $\pm$ 0.57‰) were found for both treatments in the SOM pool decomposed in the E range. The increase in the concentration of atmospheric CO_2 had no effect on the ^{15}N distribution in the SOM pools.

Portions of C_{FACE} and N_{fert} in organic matter of different pools. After three years of the impact of the elevated CO_2 concentration, the content of C_{FACE} in the soil reached 7% of the total C in the SOM, which corresponded to 1524 $\pm$ 68 kg/ha (Fig. 3, table). The total C_{FACE} in the SOM pools was distributed as follows: 231 $\pm$ 94, 184 $\pm$ 19, 776 $\pm$ 55, 262 $\pm$ 91, and 76 $\pm$ 13 kg/ha for the A, B, C, D, and E ranges, respectively (table). The mean residence time (MRT) of the C_{FACE} was calculated from the content of new C in the pools and the three-year-long impact of the elevated CO_2 concentration. The content of C_{FACE} in the SOM pools and the MRT value were independent of the thermal fractionation. The SOM pool mineralized at 200–310°C contained 4% C_{FACE} with the MRT = 94 $\pm$ 20 years; the portion of new C in the pool decomposed at 310–400°C was 21% with the MRT = 13 years.

The renovation of N in the SOM due to the application of mineral fertilizers for two years was 3.3 and 3.6%, or 76 $\pm$ 6 and 83 $\pm$ 5 kg/ha, for the treatments with the ambient and elevated CO_2 concentrations, respectively (Fig. 4). The highest content of N_{fert} was found in the SOM pool mineralized at 310–400°C. The organic matter contained 31 $\pm$ 1 kg/ha N_{fert} in the soil at the ambient CO_2 concentration and 31 $\pm$ 1 kg/ha N_{fert} in

the soil under the elevated CO_2 concentration. The lowest content of N_{fert} (about 1 kg/ha) was found in the organic matter of the pool decomposed at the highest temperature (480–1000°C). Similar MRT values of new N (about 60 years) were obtained for the initial soil and the SOM pools mineralized in the A, B, and D ranges. The longest MRTs of N_{fert} were obtained for the SOM pool decomposed at 480–1000°C (1583 $\pm$ 590 and 2695 $\pm$ 1670 years in the treatments with the ambient and elevated CO_2 concentrations, respectively). In spite of the long MRTs, no differences in the renewal of N in the pool decomposed at the temperature above 480°C were found between the treatments with the ambient and elevated CO_2 concentrations ($P > 0.05$).

RESULTS AND DISCUSSION

Thermal fractionation of SOM. The thermogravimetric analysis revealed no reliable differences in the weight loss from the soil samples between the treatments with the ambient and elevated CO_2 concentrations. This allowed identical temperature ranges to be selected for the SOM fractionation in both treatments. Based on the weight losses from the soil samples under gradual heating, five temperature ranges were selected. In the first range (A, up to 200°C), the weight loss was due to the evaporation of bound water and small amounts of volatile organics [24, 39]. The water consisted of the moisture adsorbed from the atmosphere and the hygroscopic water of salts [12, 47]. The organic substances mineralized below 200°C mainly included low-molecular compounds such as free amino acids and carboxylic acids, whose decomposition points are no higher than 150°C [20, 23, 47].

The largest losses in C and N were recorded in the B (200–310°C) and C (310–400°C) ranges. According to Kristinsen [23], the organic substances decomposed below 310°C mainly included materials of plant origin such as cellulose. A similar conclusion was drawn by Herrera et al. [16], who found from the dTG data that cellulose and hemicellulose made up the major portion of weight loss in the range from 200 to 350°C. In the B and C ranges, the decomposition of polysaccharides and the decarboxylation and dehydration of hydroxylic aromatic structures also occurred [6].

The carbon losses in the D range (390–480°C) made up 4.5–5% of the total C in the soil. The nitrogen losses in the same range were larger: 12.5–13% in the treatments with the ambient and elevated CO_2 concentration in the atmosphere. Schulten and Leinweber [37] also observed larger losses from the nitrogen-containing compounds than from the carbon-containing ones at 390–480°C. The mineralized organic substances mainly consisted of aromatic compounds such as lignin, humic substances, and kerogens [23, 27, 29, 40].

In the last (E) range (480–1000°C), the SOM losses were very small. The major part of the lost material consisted of inorganic compounds, including the decomposition products of clay minerals, and constitutional water [38].

The isotopic composition of C and the portion of new C_{FACE}. Although the total C in the soil under an elevated concentration of CO_2 in the atmosphere did not reliably increase, the C isotopic composition in the treatment with the high CO_2 concentration increased, which indicated the input of new C into the SOM. According to Leavitt et al. [26], the three-year-long period of free-air CO_2 enrichment was insufficient for the accumulation of new carbon in amounts reliably different from the C content in the control soil under the conditions of natural variation of $\delta^{13}C$. Data on the isotopic composition of the initial soil and SOM pools with different thermal stabilities are given in Fig. 2. It can be seen that the depletion of ^{13}C in the soil occurred at the expense of the pools mineralized at 310–1000°C. At the same time, the $\delta^{13}C$ values of the SOM pools decomposed at 20–200 and 200–310°C did not reliably change under the effect of the elevated CO_2 concentration.

The content of new C_{FACE} in the SOM was calculated from the difference in the C isotopic composition between the treatments with the ambient and elevated CO_2 concentrations. After the enrichment of the atmosphere with carbon dioxide for three years, the portion of C_{FACE} in the initial soils became 7%. Leavitt et al. [26] found 6.3% new C in the 0- to 15-cm soil layer in an experiment with winter wheat after enrichment with an elevated CO_2 concentration for two years, which agreed with our results. In experiments with cotton grown under an elevated CO_2 concentration for three years, the mean content of carbon brought into the SOM varied in the range of 6–12% [25]. Van Kessel

The contents of C_{FACE} and N_{fert} (kg/ha ± standard error) in the initial soil and the SOM pools with different thermal decomposition stabilities after free-air CO_2 enrichment for three years and fertilization for two years

Temperature range, °C	Treatment	C_{FACE}, kg/ha		N_{fert}, kg/ha	
20–200	A			6 ± 1	a
	B	231 ± 94	a	5 ± 1	
200–310	A			27 ± 3	b
	B	184 ± 19	a	30 ± 2	
310–400	A			31 ± 1	b*
	B	776 ± 55	b	36 ± 1	
400–480	A			10 ± 1	a
	B	262 ± 91	a	10 ± 1	
480–1000	A			1 ± 0	a
	B	76 ± 13	a	1 ± 0	
Initial soil	A			76 ± 6	c
	B	1524 ± 68	c	83 ± 5	

Notes: (A) soil at the ambient CO_2 concentration; (B) soil at the elevated CO_2 concentration.

The reliability of the differences ($P = 0.05$, capital letters) is given for the temperature parameter. The C_{FACE} values can be obtained only for the treatment with the elevated CO_2 concentration; therefore, the CO_2 parameter was not included in the statistical analysis.

*A reliable difference between the N_{fert} for the CO_2 parameter was obtained only in the SOM pool mineralized at 310–400°C.

et al. [43] observed a significantly higher content of new C (22–26%) arriving to the SOM under a meadow with *Lolium perenne* and *Trifolium repens* during an equal time period. However, the intensive input of new C into the SOM was mainly due to the higher translocation of C assimilated by the herbaceous plants, as compared to wheat, to the soil and the absence of tillage, which enhances the mineralization of SOM [43].

The percentage of C_{FACE} did not correspond to the absolute input of new C to the SOM pools; therefore, the C_{FACE} contents were recalculated in terms of kg of C/ha. The tabulated data indicate that the SOM pool mineralized in the B range had the highest content of organic C, but the portion of new C_{FACE} was smaller than in the other pools. This was mainly related to the fact that the difference in $\delta^{13}C$ between the treatments with the ambient and elevated CO_2 concentrations at 200–310°C was small, in distinction from the SOM pool mineralized at 310–400°C and containing the highest content of new C.

The MRT of the C_{FACE} in the initial soil was 40 ± 2 years, which agreed with the results of Lichter et al. [28]. In their study of the effect of an elevated CO_2 concentration on a pine forest ecosystem for six years, the MRT values were found to be 45.4, 46.8, 40.4, and

45.5 years for four SOM pools with different availabilities in the 0- to 15-cm soil layer. Licther et al. [28] used another method of SOM fractionation; therefore, they could not show reliable differences in the MRT between different pools. In another FACE experiment [41], the organic matter in a soil under a mixed forest with a similar MRT value (38.1 years) was characterized as poorly decomposable. However, significantly higher turnover rates of new C were observed for the SOM under herbaceous plants. Van Kessel et al. [43] found that the MRT of the SOM under meadows composed of *Lolium perenne* and *Trifolium repens* was 4.6 ± 1.5 and 3.1 ± 1.1 years, respectively. The MRT values of 3.5 and 15 years were obtained for the new C in experiments with *Miscanthus* [10] and corn [14], respectively.

No reliable difference in the MRT values were found between the SOM pools decomposed at low and high temperatures and between these pools and the initial soil, except for the pool mineralized at 200–310°C. Its MRT was 94 ± 20 years, which exceeded the MRT of the initial soil by 2.4 times and the corresponding values of the other pools by 3–7 times. In FACE experiments, de Graaf [5] and van Groenigen [42] estimated MRT = 9 years as the threshold value for the separation of the new and previously accumulated carbon in the organic matter of a soil under a meadow. Although the MRT values in our experiment were higher than the threshold value of 9 years, a significant input of new C into the SOM was observed. The mean residence time of the C in the SOM pool mineralized at 310–400°C was 13 years, which corresponded to the relatively high turnover rates of SOM in the experiments of other authors [14]. However, it is notable that the absence of a reliable difference in the C MRT of pools with different thermal stabilities indicates the similar availability of the SOMs for microbial degradation.

Portions of N_{fert} in SOM pools with different thermal decomposition stabilities. The FACE experiment was performed using ^{15}N-labeled fertilizers ($\delta^{15}N = 333.8‰$), which allowed the amounts of N_{fert} arriving to the different SOM pools to be calculated. The statistical analysis of the data showed no reliable difference in the N_{fert} input to different SOM pools between the treatments with the ambient and elevated CO_2 concentrations in the atmosphere. This indicated the absence of a reliable effect of the atmospheric CO_2 on the transformation of N in the soil for three years. The results of other studies gave no unambiguous answer to the question of the effect of the increase in atmospheric CO_2 on the nitrogen cycle. Dijkstra et al. [7] and van Kessel et al. [43] also found no reliable effect of the elevated CO_2 content on the dynamics of soil N. Daepp et al. [4], Hartwig et al. [15], and Zanetti et al. [46] observed the deceleration of N transformation in a soil under an increased concentration of atmospheric CO_2. However, an increase in the N turnover rate in the soil was observed in the studies of the effect of elevated CO_2 concentrations on the symbiotic N fixation [17, 34, 46].

The $\delta^{15}N$ values of the SOM pools mineralized at 310–400 and 480–1000°C reliably differed from each other and from those of other pools. The $\delta^{15}N$ of the pool with a medium thermal stability (310–400°C) was closer to the $\delta^{15}N$ of the labeled fertilizers. The $\delta^{15}N$ of the most thermally stable SOM pool (480–1000°C) did not differ reliably from the $\delta^{15}N$ of the initial soil before fertilization, which indicated the low N turnover rate in the thermally stable pool.

Two years after the application of the labeled nitrogen fertilizers, the portion of N_{fert} in the initial soil with the ambient and elevated CO_2 concentrations was 3.4 and 3.6%, respectively. This corresponded to 76 and 83 kg N/ha in the upper 0- to 10-cm layer or 27–30% of the fertilizer nitrogen applied for two years. A similar portion of N_{fert} remaining in the soil (27%) was found by MacDonald et al. [31], who studied the behavior of ^{15}N-labeled fertilizers under different agricultural crops. Significantly lower N_{fert} values (8–9%) were obtained by van Kessel [43] in a soil under meadow plants in a 10-year-long FACE experiment. However, the author attributed such a low portion of fertilizer nitrogen remaining in the soil to the large N loss due to the leaching and denitrification processes.

Similar turnover rates of new N in the SOM pools mineralized in a wide temperature range from 20 to 480°C were observed for the treatments with the ambient and elevated CO_2 concentrations in the atmosphere (MRT = 60 years). Only in the most thermally stable SOM pool (480–1000°C) did the mean residence time of N exceeded 1500 years in both treatments. Based on the ^{15}N content in the soil under an alpine meadow, Gerzabek et al. [13] found that about 50% of the N applied accumulated in the slowly decomposed SOM pool with MRT = 95 years after fertilization for five years. In our experiment, the most thermally stable SOM pool containing the smallest amount of N_{fert} with an MRT of about 1000 years can be classified as a pool of inert organic matter.

However, the absence of reliable differences in the C and N isotope labels between the SOM pools with different thermal decomposition stabilities disproves the advanced hypothesis about the correlation between the thermal stability of organic matter and its availability for microbial degradation.

CONCLUSIONS

The paper presents the first attempt to combine thermogravimetric analysis and the isotope indicator method for studying the transformation of C and N in soil under elevated CO_2 concentrations in the atmosphere. In spite of the absence of reliable differences in the total C and N between the soils with the ambient and elevated CO_2 concentrations, the application of ^{13}C-depleted carbon dioxide and ^{15}N-labeled fertilizers

allowed tracing the transformation of C and N in the SOM under spring wheat. After free-air CO_2 enrichment for three years and fertilization for two years, the content of C_{FACE} in the 0- to 10-cm soil layer reached 2167 ± 96 kg/ha and the N_{fert} content became 76 ± 6 and 83 ± 5 kg/ha in the treatments with the ambient and elevated CO_2 concentrations, respectively. The thermogravimetric analysis can be used to study the quality of organic matter in the soil by separating its organic and inorganic components and determining the ratio between the aliphatic and aromatic organic compounds in the SOM. The fractionation of soil organic matter according to the thermal decomposition stability is largely indicative of the chemical properties of SOM pools, but it does not reflect their biological properties, as was shown by isotopic analysis. The C and N isotope ratio and the calculated mean residence time of these elements in the SOM showed that the thermal stability of the organic matter pools did not reflect their availability for microbial degradation.

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